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Scanning Electron Microscopic Study On The Cocoon Filaments And Degummed Fibers Of Two Silkmoth Hybrids Of Bombyx Mori Linn

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Abstract:

The cocoon filaments and degummed fibers of CSR2 x CSR4 (Bivoltine hybrid) and PM x CSR2 (Multivoltine x Bivoltine hybrid) of Bombyx mori were examined under a scanning electron microscope (SEM). The degummed fibers obtained from the different methods used earlier, were under taken to study the characteristics of degummed fibers. The morphological study revealed that the diameter of the cocoon filament was 15 µm in diameter in PM x CSR2 hybrid and 12 µm in CSR2 x CSR4 hybrid, when studied at ultra structural level. In all the degummed process, the diameter of the degummed fiber of both the hybrids were 6 µm except soap soda degumming method, where the diameter of degummed fiber was measured 6.3 µm and the difference was due to the soap attachment to the degummed fiber. The degummed fibers were noticed intact in soap soda and 1% Na_2CO_3 methods. However, in Gulrajani method, the fibers were noticed fibrillation of 1.2 µm diameter which may be due to the more time taken for degumming process.

Key words: *Cocoon filament, Degummed fiber, Silkmoth hybrids, Bombyx mori, SEM.*

1.Introduction

The silkworm, Bombyx mori Linn., is an insect of economic importance which feeds on mulberry leaves during its larval period and spin silk cocoon. During the course of silk fiber formation, the silk is synthesized in the silk glands and converted into fiber with which the cocoons are formed. The silk fiber is a protein composed of two types of proteins, viz., fibroin and sericin. The fiber is a natural polyamide fiber with a chain of high molecules of condensed α-amino acids. It also contains small quantities of carbohydrate, wax and inorganic components, which play a significant role as structural elements during the formation of silk fiber. Each strand of silk fiber is a double structure with two parallel fibroin filaments coated by sericin. Fibroin is the central core of the natural silk gel surrounded by external sericin, without mixing due to the presence of wax material between fibroin and sericin layer (Shimizu 2000).

A single fiber is composed of several layer of fibrils i.e., macro-molecules (that combines to form the basal fibril), basal fibril, micro fibril, fibril and large fibril. The basal fibril (10~30 Å) is a bundle of macromolecules made up of ten mutually parallel molecules in straight chain arranged at equi-distance and to a large degree mutually stable. The micro fibrils (100~500 Å) are packed together to from the fibril bundle and several fibril bundles produce a single strand. The micro fibril made up of several elementary (basal) fibrils arrange in parallel fashion. Formation of micro fibril depends upon the action of inter-molecular strength in respect of the elementary fibrils and the longitudinal linkage of the macro-molecular chain that runs through more than two elementary fibrils. Fibril (1000~5000 Å) is formed by essentially parallel bunching of the micro fibrils. Formation of fibrils also depends upon the inter-molecular strength as well as the longitudinal linkage of macro-molecular and many micro fibrils arranged together to form a fibril (Fig. 1). The large fibril or macro fibril (15000 Å) is the sheath of a macro-molecule formed by the tying up of several fibrils and the fiber is formed from the piled-up large fibrils (Rui, 1998).

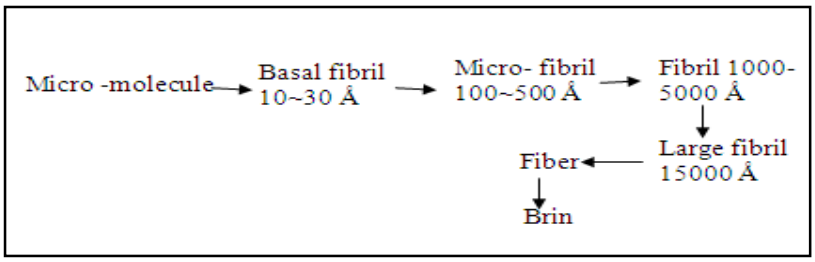

Figure 1: Flow chart of fibroin brin formation

Fibroin is the structural protein and has wide application not only in textile industry but also in non-apparel industry due to its unique properties. Silk degumming by chemical methods not only dissolves sericin but also affects in more or less pronounced decomposition of fibroin as it is unstable in many chemical reagents. Therefore, it is necessary to take proper care and attention while degumming the silk cocoons. Proper degumming of cocoon shell is very much essential and important to obtain the intact fibers. There are different degumming methods in practice for degumming the raw silk and the literature reveals that no one has elaborated the structural changes while degumming the cocoon fibers, therefore, it is felt, to study the structural changes occurred in different methods of degumming process of silk cocoons. In light of the above, the present study was conducted on the surface structure of cocoon filaments and degummed fibers of two silkmoth hybrids of Bombyx mori.

2.Materials And Methodology

The samples of the cocoon filaments and degummed fibers of CSR2 X CSR4 and PM x CSR2 hybrids of B. mori were sliced into small pieces of 2 mm sizes, with the help of a sharp blade. Three samples were taken from each hybrid from the middle part of the cocoons for morphological observations of the cocoon filaments before degumming. However, to obtain the degummed fiber, the adhesive protein i.e., sericin was removed from cocoon filament by Soap soda (Anonymous 2003), Gulrajani method (1988), 1% Na2CO3 (Mondal et al 2007a) and 105 min pressurized degumming methods and degummed fibre was used for SEM study. The samples obtained from both the cocoon filaments and degummed fibers of both the hybrids were mounted onto copper stubs using double side sticking tape. The mounted specimens were immediately coated with 20 nm thickness of gold in a Sputter coater (EMS – 550) to minimize charging under the electron beam. The gold coated samples were observed using scanning electron microscope (SEM) (JEOL 100 CX II ASID 4D, Tokyo Ltd., Japan) under an accelerating voltage of 20 kV with a beam current around 0.1 nÅ. The photographs were taken at different magnifications for morphological study of cocoon filaments and degummed fibers.

3.Results And Discussion

The fibrous protein is the fiber of raw material in silk reeling industry and the sericin is that which surround the fibroin strands. The micro-structure of silk fibers before and

after degumming was examined under scanning electron microscope (SEM). The morphological changes in the cocoon filaments and degummed fibers are shown in figures 2 and 3. The results of the present study revealed that the surface of cocoon filaments of both hybrids were smooth and the cocoon filaments consist of two layers i.e., fibroin – inner layer, which is coated by the outer sericin layer. Further, the unit cell dimension of cocoon filaments before degumming was 15µm in PM x CSR2 and 12µm in CSR2 x CSR4 hybrids (fig. 2a, 2b). It was also observed that there was significant difference of cocoon filament diameter between the bivoltine hybrid and multivoltine x bivoltine hybrids (Figs. 2a, 3a, 2b, 3b). The surface study of cocoon filaments reveals that the cocoon filaments spun in the form of α- shape and arranged in antiparalal manner. Two layered of continuous silk filament of both hybrids were noticed (Figs. 3a, 3b). After degumming the cocoon shell of two hybrids by different methods i.e., soap soda method (Anonymous 2003), Gulrajani (1988) methods (cocoons were autoclaved for 8 hr in 4 split each with 2 hr) and 1% Na_2CO_3 method (Mondal et al, 2007a), the degummed fiber surface study was conducted and found that the diameter of the degummed fiber was 6 µm (Figs. 2c, 2d, 2g, 2h, 3d, 3e) in all the degummed process fibers except soap soda processed degummed fiber which was 6.3 µm in diameter (Figs. 2e, 2f, 3c). When the cocoon filaments of both the hybrids of B. mori were processed for degumming by the method developed by Gulrajani (1988), 1.2 µm diameter fibers were also observed in the form of fibrils (Fig. 3d) along with 6 µm diameter of degummed fibers (Fig. 3d). The soap material was also found attached on the surface of degummed fiber when the cocoon filaments were processed by soap soda method (Fig. 3c).

The silk protein fibroin is fibrous in nature forming the main silk filament content, while sericin is a sticky coating substance between the layers of fibroin. The quality of cocoons depends both on sericin and fibroin. Shamitha and Rao (2006) studied the diameter of indoor, outdoor and irradiated cocoon fibers of Antheraea mylitta under SEM and revealed that the filaments of the outdoor cocoon shell showed cross bindings and bifurcation of filaments forming an intricate network and Y-shaped structures were clearly formed. In the indoor cocoon shell, the filaments are loosely arranged and slightly visible with wider gap. The number of filaments in the outdoor cocoons was found more than that of indoor cocoons**.** The sericin content, which forms a cementing substance between the filaments, was found more in the indoor cocoons than the outdoor ones. Kobashi et al, (1994) studied the morphological character of cocoon shell and filament in Antheraea yamamai, Antheraea pernyi and their hybrids. The filament had streaks

running parallel to the filament axis on the surface of the filament in all the strains. White crystalline powder of calcium oxalate was abundant on the surface of the cocoon shell filled the gaps among the filaments. Yago et al. (1994) reported the similar results in Japanese Giant silk moth, Dictyoploca japonica. Akai et al. (1997) studied the fine structure of cocoon and cocoon filaments of African Gonometa silkmoth and reported that the meshwork of the cocoon filaments was visible and no dust was accumulated. But eventually, all filaments become dirty as though they are covered with mud. There are also spherical or polyhedral crystals ranging from 3- 10 μm in diameter seen on the cocoon surface. Large numbers of calcium crystals are deposited on the newly molted cuticle, and polyhedral crystals on the cocoon surface of silk spinning insects belonging to the Bombycidae and Saturniidae families (Akai 1976 a, b and Komatsu 1980). However in present study the filament of cocoon shell showed α-shaped structure and anti parallel. There was no crystalline powder of calcium oxalate on the surface of the cocoon filament which may be due to the different race of silkworm. Mondal et al. (2007 b) studied the cross sections of cocoon filament and degummed fiber of different breeds of mulberry silkworm and reported that the cocoon fibers were tightly packed in the cocoons of pure bivoltine breeds whereas the same filament observed loosely packed in the cocoons of pure multivoltine breed i.e., in Pure Mysore**.** Robson (1985) found that one cocoon could comprise 3,500 meter of single fiber with thickness of 15-25μ. Krishnaswami and Sundaramurthy, (1972) reported that average diameter of the bave is 15-20μ for the univoltine and bivoltine races where as for multivoltine it is 6-14μ and the length of the silk bave in multivoltine pure race is 300-400 meter, multivoltine hybrid 400-500 meter and newly evolved hybrid is 600-800 meter. When processing silk for textile use, the sericin is removed by hot water during the degumming process and single fibroin filaments are spun into a thread. The diameter of the cocoon shell of wild silk moth was 13.4 μm reported by Narumi and Kobayashi (1997a, b). Narumi et al. (1994) observed that fine structure of cocoon filaments in wild silk moth where the cocoon filament size was 10μm. Shamitha and Rao (2006) Studies on the filament of tasar silkworm, Antheraea mylitta D (Andhra local ecorace) and observed that the diameter of the silk filament in both outdoor and indoor-reared cocoons of Antheraea mylitta D (Andhra local) was 19 □m. The texture of filament has become coarse due to radiation and has ultimately resulted in the damage of silk filaments. In the present finding the diameter of cocoon filament was 15 μm in CSR2 x CSR4 and 12μm in PM x CSR2 hybrids because of mulberry silk cocoon. So it was clearly observed that the diameter of

a cocoon filament varies depending upon the spinning process, arrangement of the cocoon filament and sericin percentage of different breeds and hybrids and different races and sericin percentage of the cocoon filament (Narumi and Kobayashi 1997 b). Between the diameter of the cocoon filament of CSR2 x CSR4 (15 µm) and PM x CSR2 (12µm) there was a significant difference because of the significant difference in sericin percentage. In PM x CSR2 sericin percentage (27.1%) was more than the CSR2 x CSR4 (22.65%) studied by Mondal et al. (2007a).

Shamitha and Rao (2006) reported that presence of more cementing substance (sericin) means the fewer filaments (fibrin) in the cocoon shell. In the present study, the cocoon filament diameter is more before degumming because of more sericin percentage. However, it was same and there was no significant difference in the degummed fiber diameter of both the hybrids. The cross section of raw silk is roughly elliptical. It shows the triangular twin fibroin filaments, completely covered by sericin, which normally face each other. After degumming process, the two triangular fibroin filaments (brins) separated into individual filament giving different fiber geometry from the raw silk i.e., a finer fiber and a more lustrous fiber (Sonthisombat and Speakman, 2004). Minagawa (2000) studied the detail electron microscopic structure of fibroin fiber surface of all varieties of domestic and wild cocoon by two-step replica of ethylmetaacry late carbon and reported that there is no difference in the fibroin fiber surface of both domestic and wild cocoons. Both fibrils twisted together with uneven characteristic into minute waveform structure, running in parallel with fibrous length-like striped pattern with canal. SEM observation on the cocoon shell surface revealed bundle shaped structures formed by 900-1400 fibrils (Minagawa 2000). When the brin is crushed, it splinters into numerous minute fibrils revealing the actual structure of brins. The thickness of each fibril is less than one micron and they are parallel to the axis of the fiber. A single fibril contains many microfibrils which, when examined with an electron microscope, have a diameter of approximately 100Å per microfibril. Microfibrils contain micelles, which are separated into crystalline and amorphous segments (Yong-woo Lee 1999). Gulrajani and Chatterjee (1992) reported that the fibers degummed under optimum conditions (temperature, 100°C; treatment time, 30 min; acid conc., 6.75g/l; and sulfactant conc., 3g/l) are clean, free from sericin and do not show any surface damage. As concentration and treatment time increased, fibrillation starts. Matsumura (2000) observed that the external surface of the cocoon fibers subjected to etching for 20 min at 8mÅ, 1kV AC, resultant in fibril structure of 0.1- 0.4µ. However, the etching effect under these

conditions was somewhat weak. When the etching was carried out under the same condition but with DC source the fibrils of 0.1-0.4μ were found to be parallel with one another. Moreover, fine fibril structure of 200-400μ was observed. In present study, when the cocoon shells were degummed by Gulrajani method it was found the uneven size of fiber and the fiber was not a continuous filament. As silk fiber is composed of several layers of fibrils and the microfibrils are packed together to from the fibril bundle and several fibril bundles, which produce a single strand. When degumming was carried out for a long time (8 hr.) longitudinal splitting of the fibers into finer filaments occurred. However, the action of chemicals and crushing or rubbing may easily cause longitudinal splitting of the fibers into finer filaments called fibrils. The silk fibrillation is the cause of one of the silk defects, i.e. the formation of small tufts or lousiness. Murshidabad silk fabrics degummed by alkalies (sodium carbonate, borax, sodium bi-carbonate, trisodium phosphate and disodium hydrogen phosphate) and studied the surface of filaments by SEM, which showed ruptured due to attack of alkali on fibroin (Gulrajani et al, 1990). In the present study it has been observed that soap was attached in the surface of degummed fiber processed by soap-soda method (Anonymous, 2003). Sadov et al. (1978) reported that as the part of the soap (within 1%) is stably fixed on the fiber and is not eliminated by subsequent washing, thus improving the silk by imparting to it softness and better resistance to wear. To obtain the virgin degummed fibroin for biomedical use soap attachment to the fiber is not recommendable.

Materials used	**Diameter (μm)**
1. Cocoon filament surface	
a. PM X CSR2	15.00
b. CSR2 X CSR4	12.00
2. Gulrajani (1988) method's degummed fiber	
a. PM X CSR2	6.00
b. CSR2 X CSR4	6.00
3. Soap-soda (Anonymous, 2003) method's degummed fiber	
a. PM X CSR2	6.30
b. CSR2 X CSR4	6.30
4.1% Na_2CO_3 (Mondal et al, 2007a) method's degummed fiber	
a. PM X CSR2	6.00
b. CSR2 X CSR4	6.00
SE±	0.273
CD5%	0.819

Table1: Diameter of cocoon filaments and degummed fibers

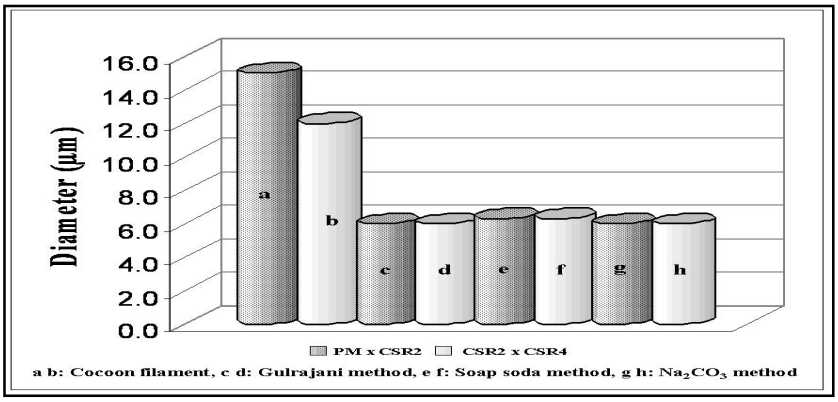

Figure 2: Diameter Of Cocoon Filament Before And After Degumming

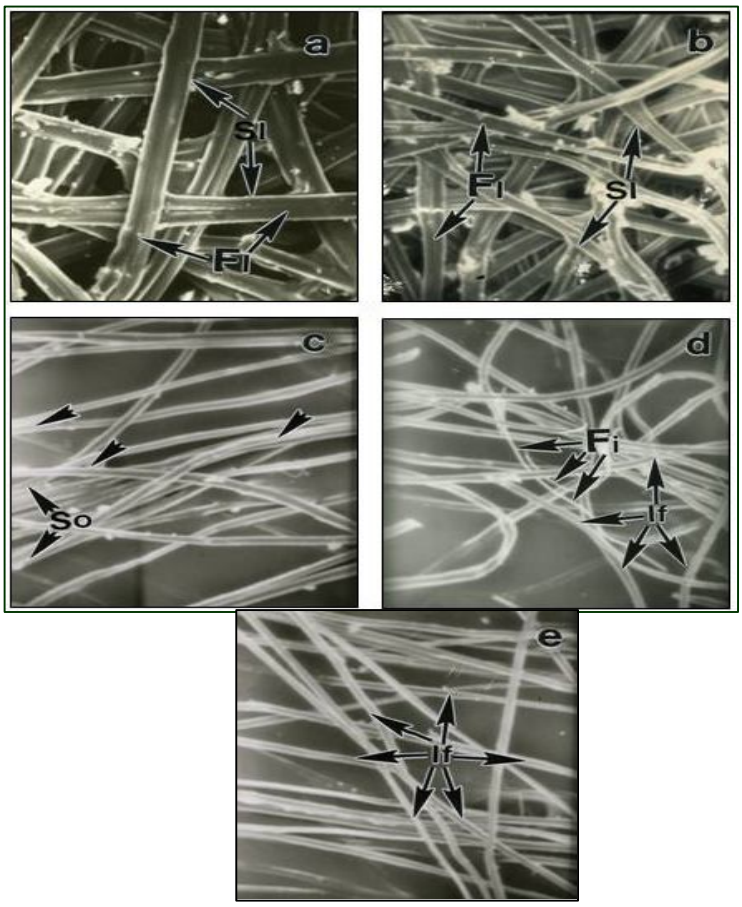

Figure 3: SEM photographs of cocoon filaments and different process degummed fibers

- a: Cocoon filament of PM x CSR2 coated with sericin layer (Sl) and fibroin layer (Fl)
- b: Cocoon filament of CSR2 X CSR4 coated with sericin layer (Sl) and fibroin layer (Fl)
- c: The soap (So) attached with degummed fiber of CSR2 X CSR4 by soap-soda method.
- d: Magnified view of intact degummed fiber (If) and fibril (Fi) of CSR2 X CSR4 hybrid by Gulrajani (1988) method.

- e: Intact fiber (If) of CSR2 X CSR4 obtained from the 1% Na_2CO_3 degumming method.

  (Marked wrongly on photograph)

4.Conclusion

It is concluded from the present findings that the diameter of cocoon filament was 15 µm in CSR2 x CSR4 and 12 µm in PM x CSR2 hybrids and after degumming, the diameter of degummed fiber was 6 µm in all the process of degummed fiber except soap-soda processed degummed fiber, where the diameter was measured 6.3 µm and it is because of the part of the soap soda attached on the fiber. The difference in diameter of cocoon filaments is due to difference in sericin content. However, in Gulrajani method the fiber starts fibrillation and the method is not suitable to obtain the intact fiber. Usually soap-soda methods are suitable for reeling industry because attachment of remaining soap to the fiber improved the silk by imparting to its softness and better resistance to wear. In 1% Na_2CO_3 there was no defect on the silk therefore process can be used in reeling industry whereas, production of fibroin for biomedical and value added product, use of soap/chemical is not recommended for the same.

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